



*Veterinary News and
Information From North
Carolina's Diagnostic
Laboratories*

Notes from Our Director...



● As you are probably aware our laboratory system is accredited by the American Association of Veterinary Laboratory Diagnosticians (AAVLD). In order to maintain this accreditation it is required that laboratories are evaluated by a team of AAVLD auditors at least once every 5 years. We hosted the AAVLD Accreditation Audit

Team this past July. The audit examined all processes that take place between the receipt of samples at the laboratory to the reporting of final results to our clients. The audit team visited all of our facilities, interviewed NCVDLs employees, and inspected diagnostic procedures, training records, equipment maintenance and calibration records, quality control records, test method validation data, and many more items related to diagnostic work. The audit team is currently preparing a site visit report from their visit. This report will be evaluated by the AAVLD Accreditation Committee in October at their annual meeting. We are anxiously awaiting the final report of the audit but feel confident in our internal quality system, our training process, and know the dedicated personnel in all of our laboratories strive to provide our clients with accurate and timely diagnostics and understand quality is paramount.

● We've officially broken ground on our new Rollins laboratory! After over two years of working closely with a design team, the North Carolina Department of Agriculture and Consumer Services, officially broke ground in July on the \$107 million Agricultural Sciences Center, a co-located facility that will house our new Animal Disease Diagnostic Laboratory as well as labs and offices for the Food and Drug Protection, Structural Pest Control and Pesticides, and Standards divisions. The approximately 220,000 square foot facility is projected to be completed in late 2020.

● Our Rollins laboratory recently participated in a National Animal Health Laboratory Network (NAHLN) tabletop exercise involving a simulated virulent Newcastle Disease outbreak scenario. The exercise covered the first three days of an outbreak investigation and focused on biosafety practices, laboratory capacity, enhanced biosafety conditions, outbreak responses, communications, and continuity of operations plan. The lessons learned from this exercise will help strengthen our laboratory's and NAHLN network's preparedness and ability to respond to high consequence animal disease events.



● A new species of tick has found its way to North Carolina. Recent tick surveys sent to the U.S. Department of Agriculture found the Longhorned tick (*Haemaphysalis longicornis*) on an opossum in Polk County. The tick has been previously identified in Arkansas, New Jersey, Virginia and West Virginia.



Three life stages of *H. longicornis*.
Adult female (left), partially en-
gorged nymph (center), and larvae
(right) www.fonseca-lab.com

The Longhorned tick is an exotic, East Asian tick. Prior to its identification last fall in New Jersey, the Longhorned tick was not typically found in the United States. It is a serious pest of livestock in its native regions and the means of introduction into the U.S. is unknown. Its presence in N.C. signals the need among livestock producers and residents for greater awareness, surveillance, and tick control management. It is an aggressive biter and frequently builds intense infestations on animals causing great stress, reduced growth and production, and blood loss. The tick can reproduce parthenogenetically (without a male) and a single fed female tick can create a localized population. It is a known/suspected carrier of several viral, bacterial and protozoan agents of livestock and human diseases. Known as a three-host tick, this tick can spread pathogens among a diverse host range on which it feeds.

While the Longhorned tick has not been linked to any human infection in the US, the N.C. Division of Public Health is working with NCDA&CS to understand its distribution and monitor for diseases it may carry. The finding of this tick in the state corresponds with an effort by the N.C. Department of Public Health to identify ticks in all 100 counties of the state. Veterinarians are asked to submit ticks they find on clinical patients to help track and identify tick populations in North Carolina. If you are a veterinarian practicing in North Carolina and are interested in participating in this study, email Dr. Alexis M. Barbarin at NCTickID@dhhs.nc.gov.

Jim Trybus, DVM, DAVCP



www.fonseca-lab.com

Bovine Respiratory Syncytial Virus

By: Alison Tucker, MA, VMD, DAVCP

As cooler weather approaches, increased movement of cattle, including through sales, results in outbreaks of bovine respiratory disease complex (shipping fever). This is a multi-factorial disease complex associated with one or more infectious agents, stress and impaired or incomplete immunity. Bovine Respiratory Syncytial Virus (BRSV) is a virus that causes respiratory disease in cattle world-wide and is a component of enzootic pneumonia of calves and of shipping fever. Calves that survive pneumonia frequently have reduced growth and failure to gain. Losses for the producer include death losses, reduced growth, and treatment costs.

BRSV can occur in cattle of any age but calves under 6 months of age are most severely affected. Infection and disease can occur even in the face of maternal antibodies.

Infection can come from aerosol droplets and direct contact with infected cattle. Infection can also come from contaminated surfaces such as buckets, fences, trailers and feed tubs.

Response to viral infection varies from severe respiratory disease to very mild clinical signs. The incubation period is 2 – 5 days. Initially infected calves are febrile with ocular and nasal discharge and with variably severe respiratory distress. Viral replication occurs in respiratory epithelium and in macrophages. Viral clearance is by T cell mediated cell death of infected cells. This begins at 8 days post infection (dpi) and virus can be completely cleared from the lungs by 15 dpi. During this period airway clearance mechanisms are compromised and secondary bacterial infection is common. While the virus is cleared quickly, secondary bacterial pneumonia can be protracted. Currently no commercial products are available to cure BRSV and therapeutic strategies focus on ameliorating clinical signs and controlling secondary bacterial infection.

Outbreaks typically occur in the fall and winter in North Carolina and are associated with movement of cattle, particularly calves, and mixing of animals from multiple sources. Confirmed cases seen at the NCVDLS over the past few years had the following features:

- Age ranged from 3 weeks to 8 years but 75% were <1 year of age.

- 75% were beef breeds.

- All cases had multiple animals in the herd affected.

- 50% of the affected herds claimed to have had additions to the herd within the previous 11 – 28 days.

- 70% of herds were unvaccinated.

- Co-infections included *Mannheimia hemolytica*, *Mycoplasma sp* and *Histophilus somni*.

- Bovine viral diarrhea virus was present in <15% of submissions tested.

- Infectious bovine rhinotracheitis was not present in any submissions tested.

Diagnosis of the disease within the NCVDLS is based on history and clinical signs reported by the submitters, gross postmortem examination findings, histopathology findings, and immunohistochemistry. At necropsy there is a cranioventral pneumonia with atelectasis and caudodorsal emphysema. Secondary bacterial pneumonia results in fibrinosuppurative bronchopneumonia with pleuritis.

Histopathologic findings in early cases include necrotizing bronchiolitis progressing to broncho-interstitial pneumonia with syncytia. After 8 days bronchiolar epithelial hyperplasia and alveolar type II pneumocyte hyperplasia are seen and there is loss of syncytia. The lesion can progress to bronchiolitis obliterans with atelectasis.

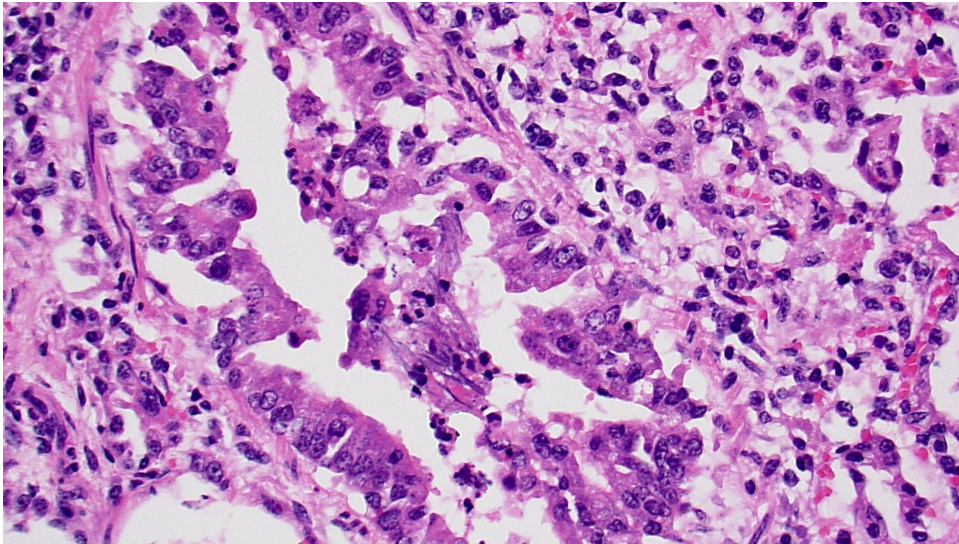


Figure 1. Calf lung with bronchiolitis and syncytia formation, H & E

Immunohistochemistry uses antigen-antibody interactions to demonstrate the presence of the virus in respiratory epithelial cells and macrophages. PCR is more sensitive than immunohistochemistry but currently is prone to false positives due to vaccination with modified live vaccines.

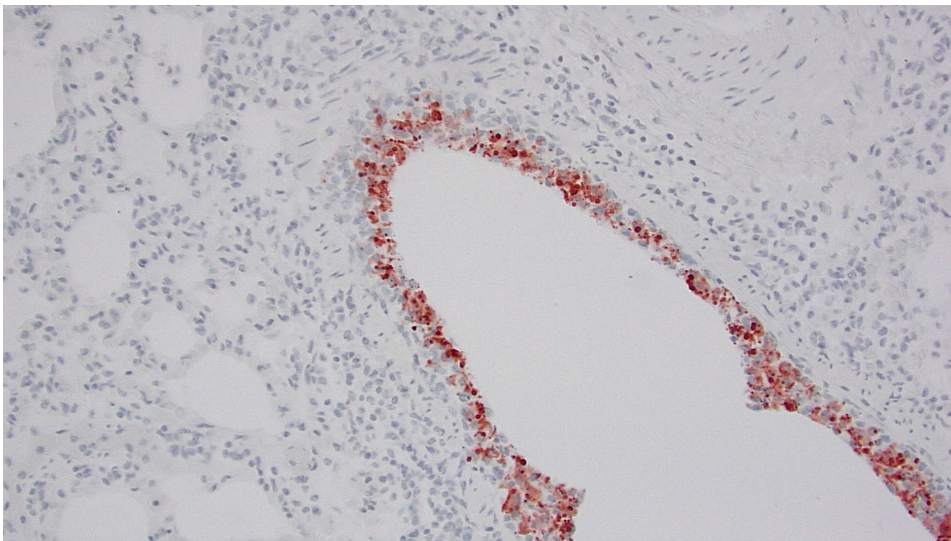


Figure 2. Calf lung with immunohistochemistry highlighting BRSV in bronchiolar epithelial cells

A variety of products are available for vaccination but complete prevention is not possible. General guidelines include timely vaccination before shipping, following vaccination protocols including administration of boosters, quarantine of cattle after shipping, attention to hygiene and careful monitoring of calves to catch early cases. Producers are encouraged to work with their veterinarians to develop protocols to help minimize the impact of BRSV and other components of the bovine respiratory disease complex.

Tis the Season for EEE

By: Stacy Robinson, DVM and Alison Tucker, MA, VMD, DAVCP

The first case of Eastern Equine Encephalitis (Eastern Equine Encephalomyelitis, Sleeping Sickness) this year was in Richmond County in July. It was followed by five additional cases as of the writing of this article. One was a donkey and four were horses. Three were diagnosed in July and two were diagnosed in August. Two of the equines were from Onslow county. The fourth case was from Duplin County. The most recent cases were from Carteret County and one was from Craven County. Last year there were 6 cases of EEE reported in North Carolina by October. From 2010-2016 the number of cases ranged from 2 cases to 20 cases/year. The lowest number of cases was in 2011 and the highest number was in 2012. The average number of cases per year from 2010-2016 was 9 cases per year.

Eastern Equine Encephalitis (EEE) is caused by a virus in the genus Alphavirus, family Togaviridae. It is transmitted via the bite of an infected mosquito. It is not transmitted by direct contact from horse to horse. Clinical signs that can be seen include anorexia, ataxia, blindness, head-pressing, circling, fever, seizures, somnolence, and in some cases no clinical signs are noted prior to death. Case fatality rates that are reported in horses vary from 75 % to 90%. Surviving equines are reported to still have neurologic deficits even after recovery.

Gross findings in horses with Eastern Equine Encephalitis virus infection are very minimal at best to nonexistent. In some cases, multifocal pinpoint petechiae can be seen in the white matter of the brain and spinal cord but this is not pathognomonic for the disease.

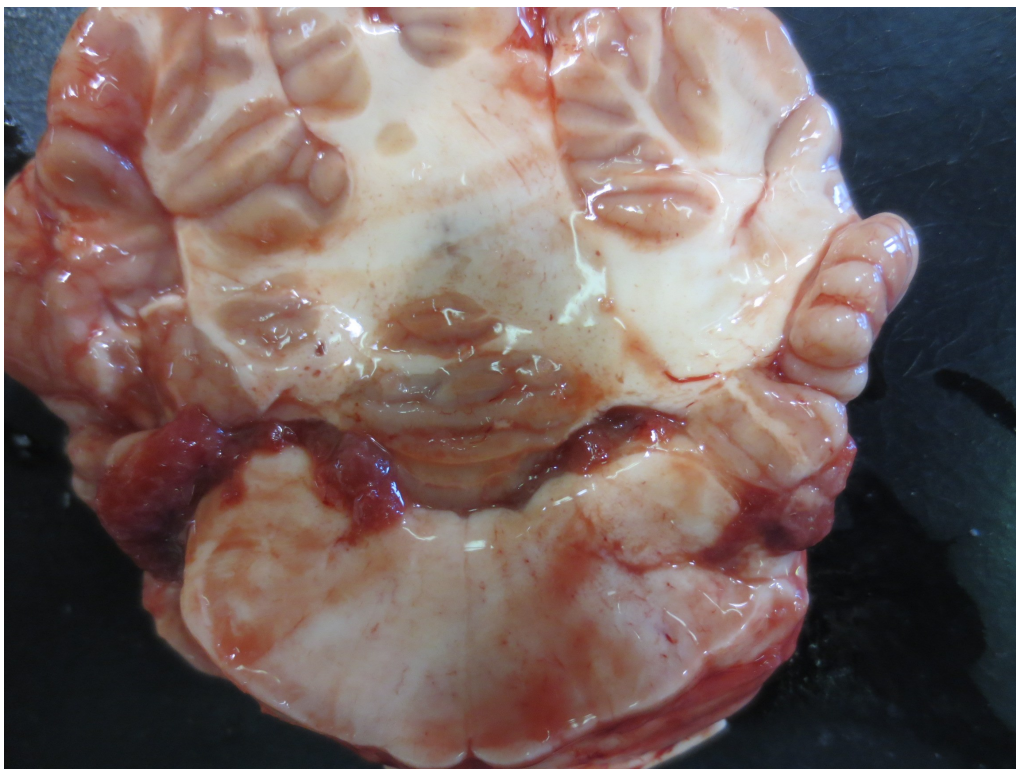


Figure 1. A few subtle multifocal petechiae in the white matter of the cerebellum and brainstem

Diagnosis is primarily made via histopathologic examination of the brain and in some cases the spinal cord with confirmation via immunohistochemistry or PCR. Therefore, the whole body of a neurologic equine may be submitted or alternatively only the head may be submitted to the diagnostic laboratory for testing. Serology (IgM antibody- capture ELISA, plaque reduction neutralization test) may also be used to make a presumptive diagnosis with compatible clinical signs. Additional information about serology testing can be obtained by contacting the National Veterinary Services Laboratory (NVSL).

Differential diagnoses for acute neurologic disease include Rabies, West Nile Virus, Herpes Virus, and hepatic disease resulting in hepatic encephalopathy.

Humans can acquire the infection via the bite of an infected mosquito. Children younger than 15 and adults older than 50 years of age are at most risk for severe clinical disease. CDC reports a mortality rate of 33% in human clinical cases. Most survivors of clinical disease have permanent neurologic deficits.

In equines, prevention is accomplished with proper vaccination. Unvaccinated horses should be vaccinated prior to the start of the mosquito season which typically runs from April through October in NC. The horse should receive a booster vaccine 4 to 6 weeks later. In some states annual vaccination thereafter is adequate. In North Carolina it is recommended to booster every 6 months. Following proper vaccination protocol is extremely important. Three of the cases this year had received one vaccine against EEE 6 to 8 months prior but never received a booster vaccine. In one case the animal was never vaccinated against EEE. In two cases this year, the animal had been vaccinated the previous year but had not received a vaccine during the current year. Failure to follow proper vaccination protocol and keeping horses current on the vaccine has been associated with cases in NC in previous years as well.

For both horses and humans, it is recommended to reduce mosquito breeding grounds such as areas of stagnant water (flowerpots, bird baths, etc.), remain inside during primary mosquito feeding times and use appropriate mosquito repellents.

The Virus Behind the Scenes...

As mentioned above, Eastern equine encephalitis virus (EEEV) is an Alphavirus of the family Togaviridae. Along with other members of the genus such as Western equine encephalitis virus (WEEV) and Venezuelan equine encephalitis virus (VEEV), EEEV requires an arthropod vector. For EEEV, birds, particularly passerines, are the amplifying hosts. Mosquitos are the vectors and areas of freshwater swamps carry a higher risk. The mid-Atlantic and southern United States are endemic areas but the range has increased widely and cases are reported in the northeast and upper Midwest as well. *Culiseta melanura* is the principal endemic vector but these mosquitos do not feed extensively on mammals. A variety of species of mosquitos are bridge vectors between amplifying hosts and other species. Humans, horses and other equids, new world camelids, sheep, cattle and deer have encephalitis; rare cases of encephalitis have also been reported in dogs and cats. In birds, the virus can target multiple organs and cause disease including encephalitis, myocarditis or enteritis (ratites). In birds such as the amplifying hosts infection can be asymptomatic.

The incubation period reported in the literature for EEEV varies but is generally considered to be between 2 and 10 days post infection (dpi) for horses. The virus replicates in regional vessels and lymph nodes. Viremia develops, followed by secondary replication in lymph nodes and muscles. A second viremia develops and this results in hematogenous invasion of the central nervous system. Although there is viremia the level is low and the horse is not an amplifying host. Once in the central nervous system the virus replicates in neurons, glial cells and blood vessels. Neuronal cell death is assumed to be by virus-induced apoptosis, as has been demonstrated in other Alphaviruses.

Clinical signs include an initial pyrexia and depression followed by temporary recovery. The fever returns in conjunction with onset of neurologic signs. This biphasic fever is present around days 2 and 6. In many cases the initial pyrexia is not recognized and the neurologic signs are the first clinical signs observed. Neurologic signs are primarily indicative of cortical disease. They are characterized by restlessness, altered consciousness, altered behavior including both somnolence and aggression, central blindness, head pressing and terminal paralysis with recumbency. In fatal cases, the clinical progression is typically 2 – 4 days. In survivors, the neuronal cell death results in retention of neurologic deficits.

At necropsy, lesions are best characterized by microscopic evaluation of tissues. In the brain the initial inflammation is neutrophilic and there can be malacia, necrotizing vasculitis, perivascular hemorrhage and edema and perivascular cuffing. Neutrophils disappear after several days and the inflammation becomes more lymphocytic with gliosis, neuronal degeneration and neuronophagia. Perivascular cuffs become thick and are predominantly lymphocytic.

The cerebral cortex is the most severely affected and the cerebellum is least affected. Hypothalamus, thalamus and pyriform lobes are also affected. Changes can extend to the spinal cord. Using immunohistochemistry with antibodies against EEEV to localize the virus within lesions, we see that the distribution is segmental and occurs throughout the brain and spinal cord, but is not present diffusely, once the horse has developed clinical signs.

Although horses can, in few cases, survive the infection, survivors often retain neurologic deficits.

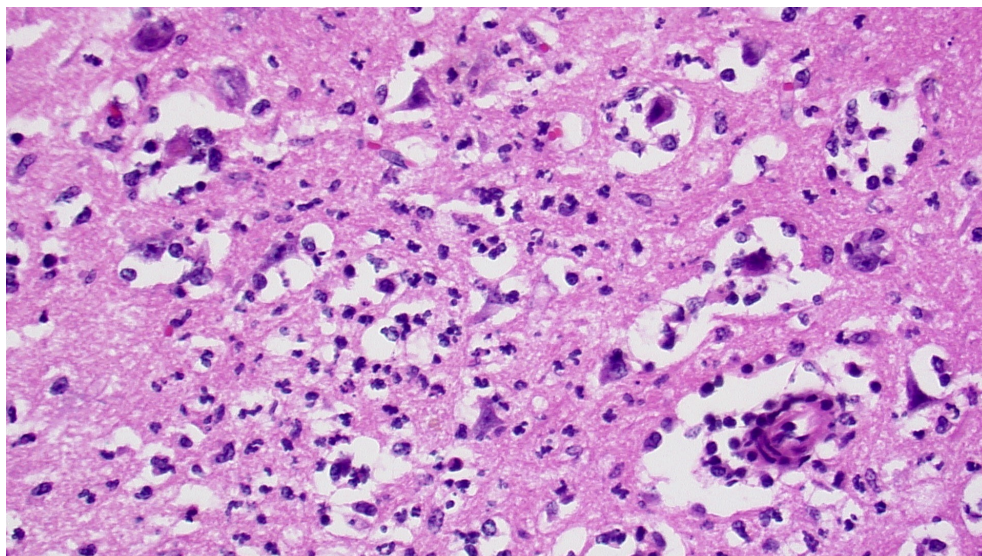


Figure 2. Cerebral gray matter showing acute neutrophilic encephalitis, H & E 20 x.

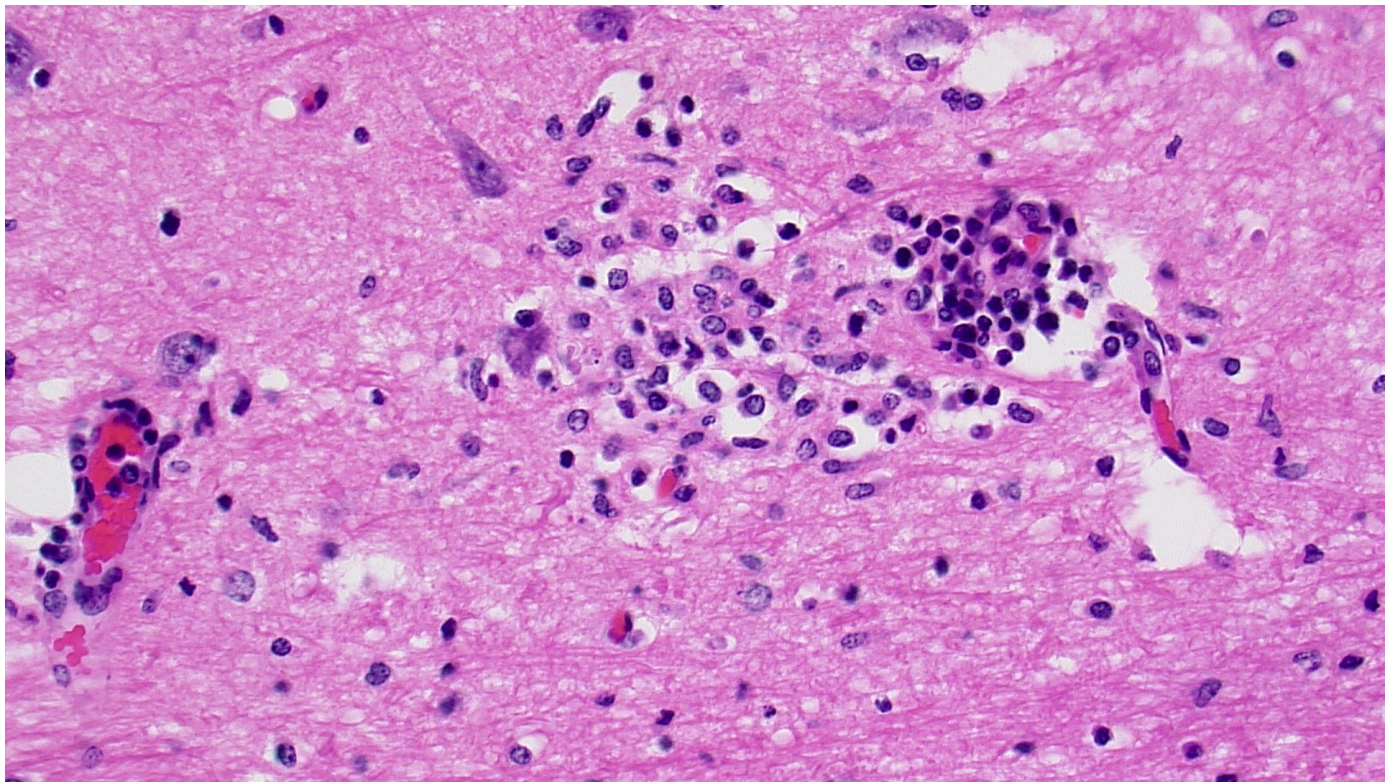


Figure 3. Subacute encephalitis with lymphocytic and neutrophilic inflammation, perivascular cuffing and neuronophagia, H & E 40 x.

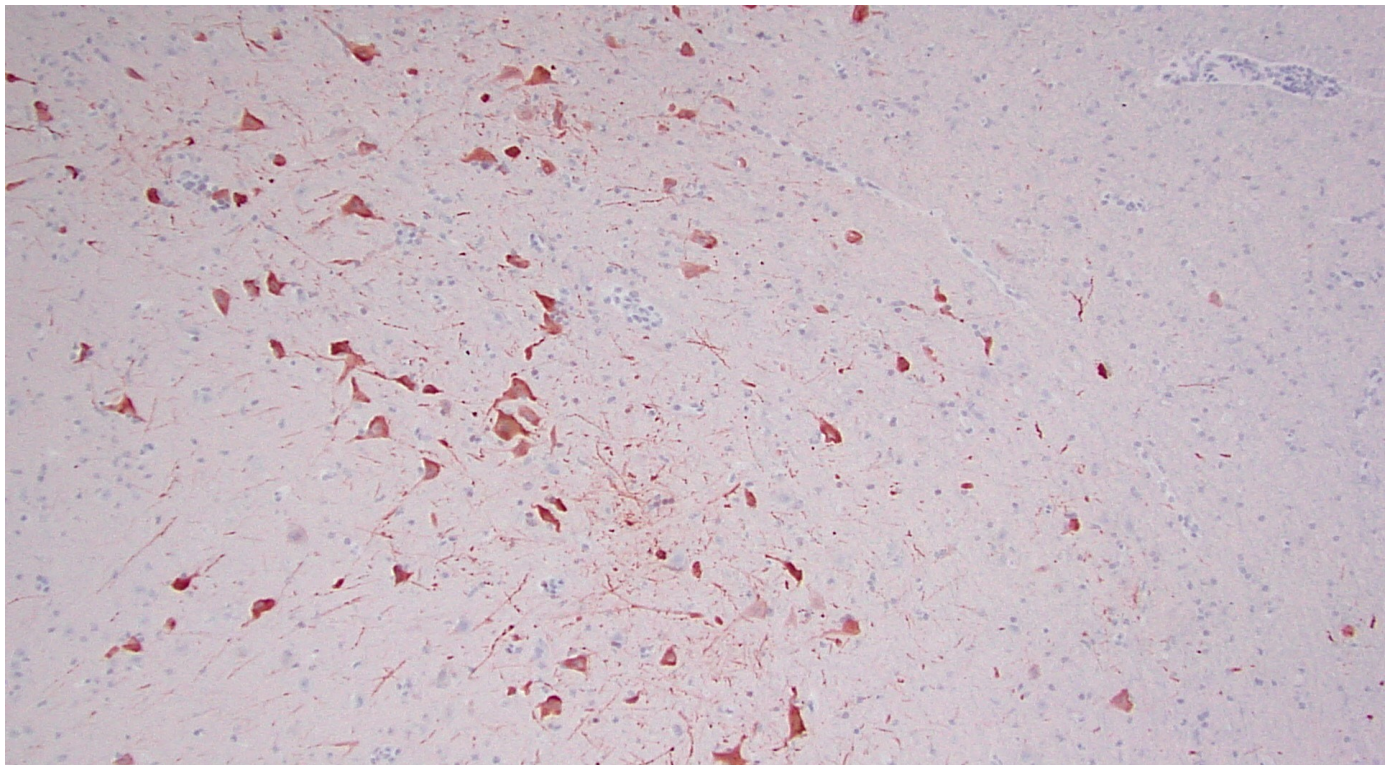


Figure 4. Cerebral gray matter showing infected neurons, IHC 20 x.

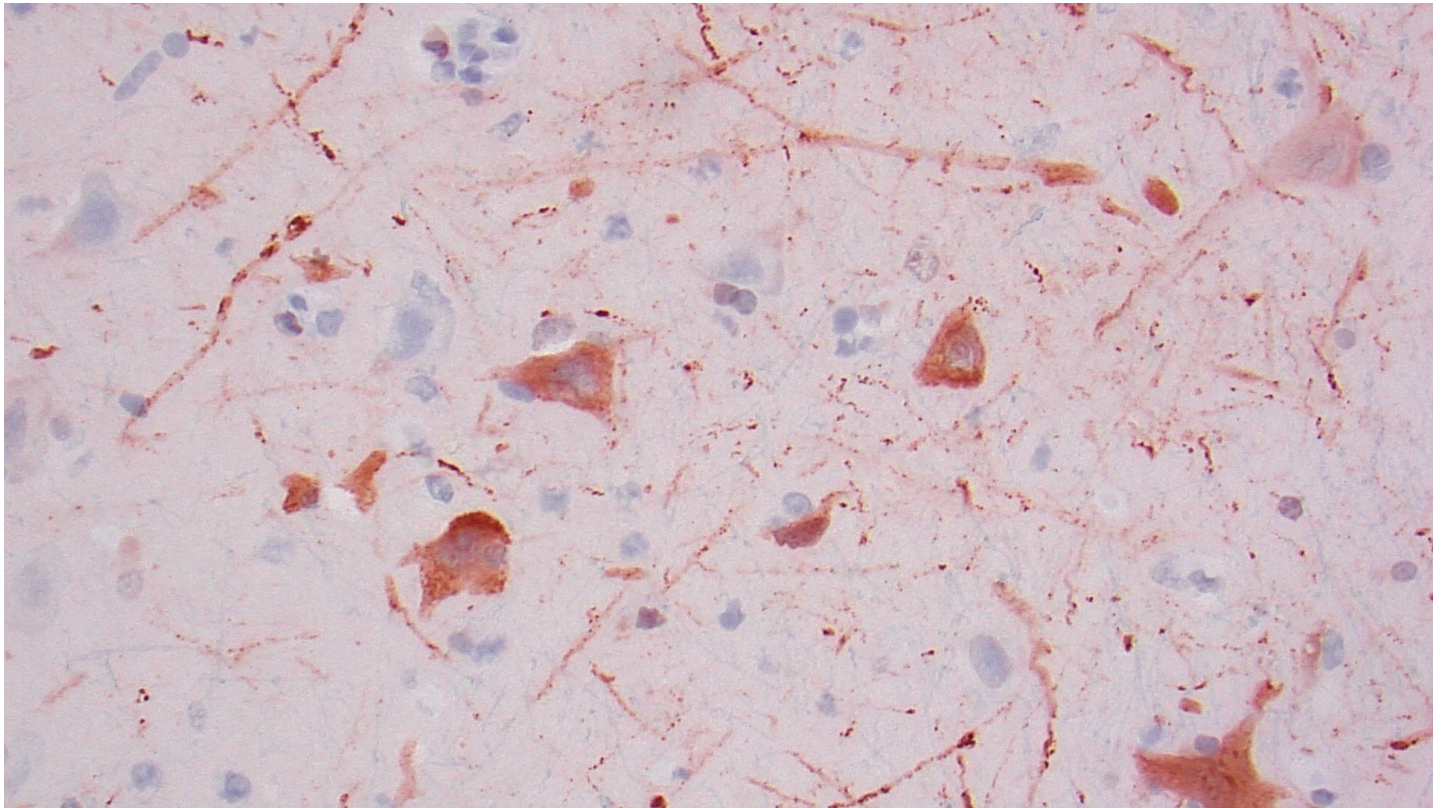


Figure 5. Cerebral gray matter with virus in neurons with axons and dendrites involved, IHC 40 x.

Sources and Further Reading:

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<https://www.cdc.gov/easternequineencephalitis/>

Intravascular Lymphoma

By: Elise Lavie, DVM

Clinical History

An 8-year-old, neutered male, mixed breed dog presented to the veterinarian with a 3 day history of possible seizures and decreased appetite, and a 10 day history of slight lethargy. The dog lived on a horse farm and had last received vaccinations in 2011. On arrival at the veterinary clinic, he was started on IV fluids, a gastroprotectant, an appetite stimulant, anti-nausea medication, anti-inflammatories, and antibiotics. He showed slight improvement over night at the hospital. When bloodwork came back the next day, he was found to have moderate renal azotemia and mildly elevated liver enzymes. An abdominal ultrasound was performed which revealed no significant findings. The patient was started on an additional antibiotic with the suspicion of leptospirosis. That evening, the dog collapsed while outside on a walk. On recovery from this episode, his right eye was bulging slightly and had an increased intraocular pressure. Blood pressure was assessed, found to be moderate to severely elevated with an average value of 190/117, and the patient was started on blood pressure medication. Later that evening, the patient had what was suspected to be a seizure which responded to treatment, however the patient continued to decline over the next 24 hours. Renal values remained elevated despite fluid administration, and blood pressure remained elevated in the face of treatment. All leptospirosis testing was negative. The patient continued to have seizures which stopped responding to treatment. He was humanely euthanized 2 days after admission to the hospital and his body was submitted for necropsy.

Gross Necropsy

Gross necropsy findings were fairly unremarkable. The liver margins were slightly rounded and the liver had an accentuated reticular pattern. The spleen was moderately enlarged with a 1.5x1.5x1.0 cm nodule in the tail of the spleen which was tan and bulged on cut surface and multiple smaller nodules palpable in the parenchyma. The anterior chamber of the right eye was red and the iris could not be completely visualized. There were multiple nodules in the pancreas. The left adrenal gland had an approximately 3mm diameter tan nodule in the medulla and the remainder of the medulla was diffusely mottled tan and brown and the medulla of the right adrenal gland was mottled tan and brown. The left adrenal gland was 2.0x1.0 cm and the right adrenal gland was 1.0x0.5 cm. Kidneys appeared grossly normal.

Histopathology

Histopathology revealed an intravascular round cell neoplasia present in the brain, heart, spleen, liver, lung, kidney, urinary bladder, adrenal gland, pancreas, intestine, and eye (all tissues sectioned). This neoplasia was most consistent with intravascular lymphoma. Additionally, the eye had moderate to marked fibrinosuppurative panophthalmitis and myositis with secondary glaucomatous changes. The liver had mild, multifocal cholestasis. Changes in the pancreas were indicative of the result of a past episode of pancreatitis. The remainder of changes noted on gross necropsy were common age-related or benign changes and of no clinical significance.

Intravascular Lymphoma

Intravascular lymphoma is characterized by the proliferation of lymphocytes within small blood vessels with limited to no extravasation of lymphocytes into the surrounding tissue. Clinical signs are the result of blockages of those small vessels by thrombosis or infarction. It is an uncommonly diagnosed disease in both veterinary and human medicine. There are a handful of case reports of intravascular lymphoma in veterinary medicine, with a very small number being diagnosed pre-mortem. It is not yet known why, but IVL does not show up on bloodwork despite large numbers of lymphocytes within the blood vessels. It is suspected that the lymphocytes lack the ability to adhere to blood vessels walls and therefore to make their way out of the blood vessels into the surrounding tissue, however this does not explain why IVL does not show up as a lymphocytosis on a complete blood count. Biopsy can diagnose IVL, however most cases which are diagnosed pre-mortem have a survival time under 2 months. In humans, IVL seems to have a predilection for the central and peripheral nervous systems or skin, however the presentation of intravascular lymphoma (IVL) can be varied depending on the organ of involvement, further adding to the challenge of reaching a pre-mortem diagnosis.

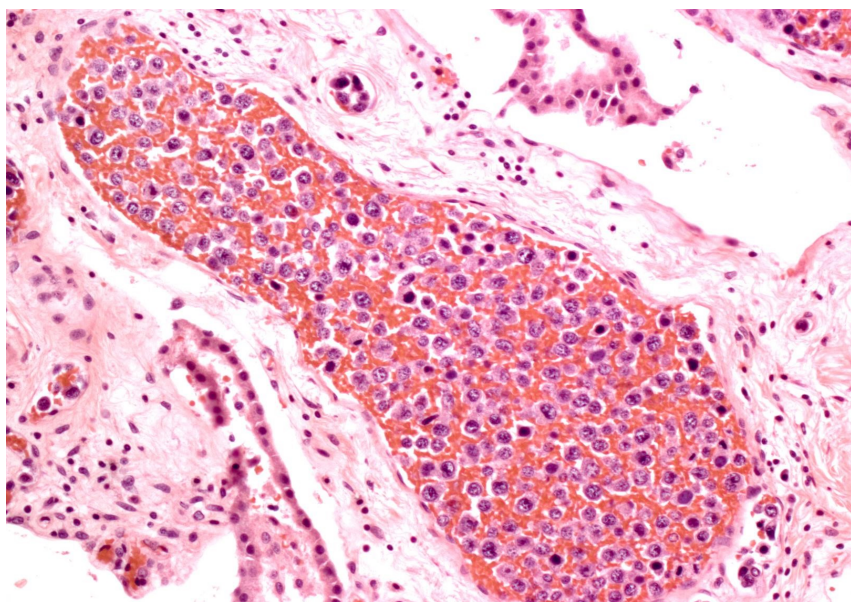


Figure 1

Brain; small vessel
filled with neoplastic
lymphocytes

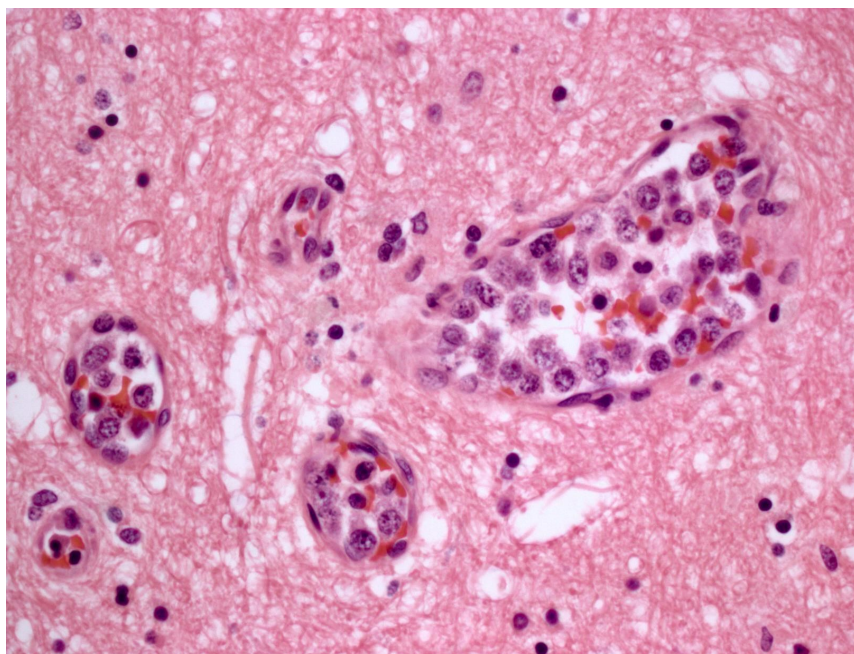


Figure 2

Brain; multiple small
vessels filled with
neoplastic lymphocytes

The NCVCLS REPORT



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Veterinary Staff

Rollins Laboratory (919) 733-3986

Director

Dr. Jim Trybus

james.trybus@ncagr.gov

Veterinary Diagnosticians

Dr. Jennifer Haugland

jennifer.haugland@ncagr.gov

Dr. Stacy Robinson

stacy.robinson@ncagr.gov

Dr. Mahogany Wade

mahogany.wade@ncagr.gov

Veterinary Pathologists

Dr. Tahseen Abdul-Aziz

tahseen.aziz@ncagr.gov

Dr. Steven Rushton

steve.rushton@ncagr.gov

Dr. Alison Tucker

alison.tucker@ncagr.gov

Dr. Allison Boone

allison.boone@ncagr.gov

Arden Laboratory (828) 684-8188

Director

Dr. Richard Oliver

richard.oliver@ncagr.gov

Veterinary Diagnostician

Dr. David Drum

david.drum@ncagr.gov

Monroe Laboratory (704) 289-6448

Director

Dr. Heather Wyss

heather.wyss@ncagr.gov

Veterinary Diagnostician

Dr. Elise Lavie

elise.lavie@ncagr.gov

Elkin Laboratory (336) 526-2499

Director

Dr. David Ackerman

david.ackerman@ncagr.gov

Veterinary Diagnostician

Dr. Jessica Kees

jessica.kees@ncagr.gov